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Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*, (Homoptera: Cicadellidae)☆

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Abstract

The ultrastructural morphology of the mouthparts of the glassy-winged sharpshooter, *Homalodisca coagulata*, and method of plant penetration was examined using light microscopy, scanning electron microscopy, and transmission electron microscopy methods. The gross morphology of the labrum, labium, and stylet fascicle was consistent with what has been described for other plant-sucking homopterans. The ultrastructural examination of the mouthparts revealed unique details that have previously gone unreported. Several types of sensilla-like structures having the form of pegs and multi-lobed objects were identified on the outer surfaces of the labrum and within the labial groove. Dendritic canals terminated in an extensive network of smaller canals at the distal tip of the maxillary stylets below a series of surface denticles suggesting that this area may have a sensory function associated with locating xylem elements of host plants. Examination of salivary sheath pathways established that 65% of the plant penetrations by this insect terminated in the xylem vessels of the host plant. Probing by the insect was largely intracellular and terminal branching of a single probe site was common. Plant surface feeding sites varied with the stage of development which correlates with the depth of the xylem vessels and the length of the maxillary stylets of the various instars.
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1. Introduction

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) has significant economic importance because it serves as a vector for Pierce's Disease in grape, leaf scorch in oleander and almond, and variegated chlorosis in citrus by transmitting *Xylella fastidiosa*. This xylem-limited bacterium causes plant degeneration and/or death through blockage of the water conducting system (Tyson et al., 1985; Purcell and Hopkins, 1996; Purcell and Saunders, 1999). Transmission of different strains of *X. fastidiosa* can cause other diseases in avocados, peaches, apricots, cherries, alfalfa and many trees and ornamentals. This insect is reported to be a xylophagous leafhopper that infects its host while gaining sustenance by inserting its maxillary stylets into the xylem elements and sucking out the sap (Anderson et al., 1989). The known host range of *H. coagulata* numbers over 100 species and 37 families, ranging from grasses to plants having woody stems (Adlerz, 1980; Hoddle et al., 2003). The adults appear to be more polyphagous than the nymphs and they appear to have a seasonal preference for certain plant hosts (Adlerz, 1980; Mizell and French, 1987; Brodbeck et al., 1990).

Plant penetration and feeding by plant sucking insects feeding primarily on phloem fluids, such as many of the aphids and whiteflies, has been an often studied behavioral phenomenon (Pollard, 1973). However, little is known about the fine structure of the mouthparts of xylophagous insects and, in particular, how the mouthpart structure relates to function in locating the xylem tissue within the plant host. The reports on morphology and fine structure of the homopteran mouthparts have mostly involved the use of light and transmission electron microscopy (Davidson, 1913, 1914; Weber, 1928; Cobben, 1978; Pollard, 1968, 1973; Spiller et al., 1985; Spiller, 1989). A few studies

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having a different focus than description of mouthpart morphological fine structure have provided selective views of the mouthpart elements associated with plant penetration and feeding using scanning electron microscopy (SEM) (Backus and McLean, 1982; Foster et al., 1983; Freeman et al., 2000, 2001; Mora et al., 2001). There is abundant data available on the feeding behavior and the damaging effects of plant penetration by other homopterans (Pollard, 1968; Sogawa, 1973; Backus, 1988; Kimmins and Tjallingii, 1985; Spiller, 1989; Ecale and Backus, 1995; Zhou and Backus, 1999; Mora et al., 2001). These studies have categorized penetration of plant tissues as intercellular, extracellular, or a mixture of both routes by microscopically examining the salivary sheath pathways secreted during feeding that remain in the plant tissues and also by using electrical penetration graphing techniques. However, there are no published reports about the external location of the feeding sites, probing behavior or salivary sheath formation within the plant tissues as the GWSS feeds throughout its development from hatching to adult. This type of information is needed for the formulation and presentation of artificial diets for the design of rearing protocols where the GWSS is to be reared for mass production of its parasites and predators for use in a biological control program. Also, this information could possibly clarify why some cultivars of the host plants are resistant to transfer of *Xylella* by this leafhopper.

Thus, the focus of this study was to examine ultrastructural morphology of the GWSS mouthparts including the labrum, labium, and the mandibular and maxillary stylets using light, transmission electron microscopy (TEM) and SEM. Determinations of the penetration paths of the stylets within host tissues of sunflower, *Helianthus annuus* L., during and after feeding were made by examining the positions of salivary sheaths using primarily optical and SEM methods. Efforts were also made to resolve whether the feeding sites and probing behavior differed as development of the insect proceeded from hatching to the adult stage with special attention to the incidence of stylet penetration which does not terminate in xylem tissue.

2. Materials and methods

2.1. Insect rearing

Egg masses of the GWSS were collected in Riverside County, CA and after hatching, the insects were maintained within cages contained in a controlled environmental chamber set at 28 °C, 60% RH and a 16L/8D photophase. The insects were allowed to feed on chrysanthemum, sunflower, and hibiscus which were replaced with new plants about every 2 weeks. Samples of the intact mouthparts, i.e. labrum, labium and stylets, from insects and their cast exuviae, in all stages of development, were collected up to two generations after the colony was initiated.

2.2. Sample preparation

Some samples were fixed and dehydrated in acidified 2,2-dimethoxypropane (DMP) (Bjerke et al., 1979). These specimens were then rinsed several times in absolute ethanol and dried in a model 810 Tousimis® critical point drier using CO_2 as the transitional fluid. Following drying, the samples were mounted on aluminum stubs and coated with Au/Pd (60:40) in a Balzers® SCD 030 sputter coater. Other samples, such as the exuviae, were air-dried and sputter coated prior to examination. The specimens were then examined and photographed with a JOEL® JSM 6300 scanning electron microscope.

Samples for TEM were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer at pH 7.4 and post-fixed in buffered 2.0% OsO_4. These specimens were then dehydrated in a graded series of acetone with two changes of 100% acetone. At the 70% dehydration step, the specimens were stained with saturated uranyl acetate. Following dehydration, the samples were infiltrated and embedded in Epon/Araldite resin. Ultrathin sections were cut using a RMC ultramicrotome, placed on formvar-coated slot grids and then stained with lead citrate prior to examination with a JOEL® JEM 100 CX transmission electron microscope.

Preparation of plant tissues for light microscopy consisted of free hand sections that were cleared in lactic acid and phenol (Cohen et al., 1998), stained in McBride's solution (Backus et al., 1988), rinsed in absolute ethanol, transferred to xylene and mounted on glass slides with Harleco® synthetic resin (Hartman-Leddon, Philadelphia). These preparations were then examined and photographed using a model SZH Olympus® dissecting microscope.

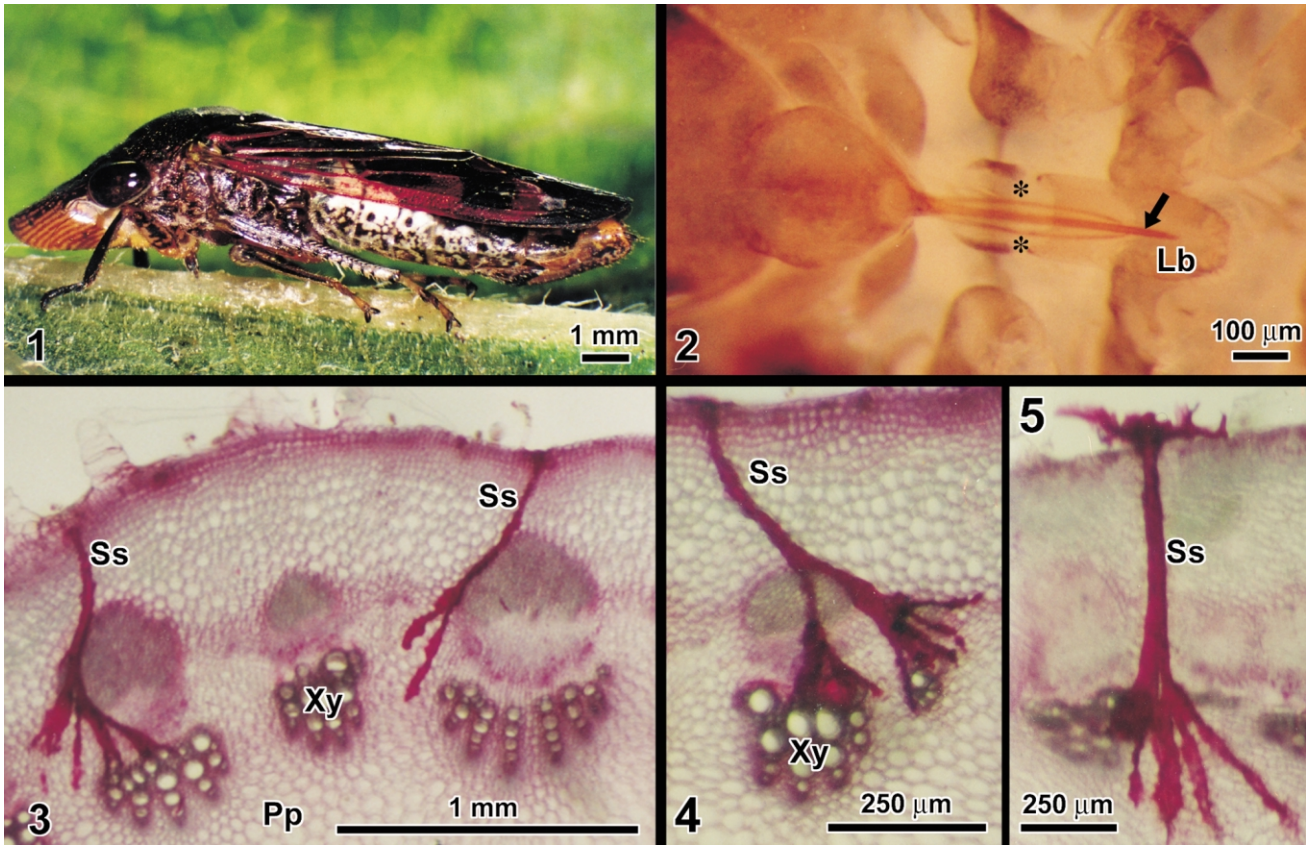
3. Results

3.1. Mouthpart morphology

The mouthparts of *H. coagulata* include the labrum, labium and a stylet fascicle consisting of two mandibulary and two maxillary stylets. The mouthpart complex is recognized as a tubular structure without accompanying labial or maxillary palpi and, in the case of the GWSS, is usually orientated in a perpendicular or slightly backward direction during feeding activity (Fig. 1). When feeding on plant stems, the insect is positioned head down towards the soil. When the insect is not feeding the mouthparts are directed backward toward the body and the stylets are usually withdrawn into the labium (Fig. 2).

3.2. Labrum

Fig. 6 shows the cone-shaped labrum (Lm) that is attached proximally to the anteclypeus (Ac) and overlies the labial groove (Lg) of the labial segments (Fig. 9). On the exposed surface of the labrum, there are numerous short,



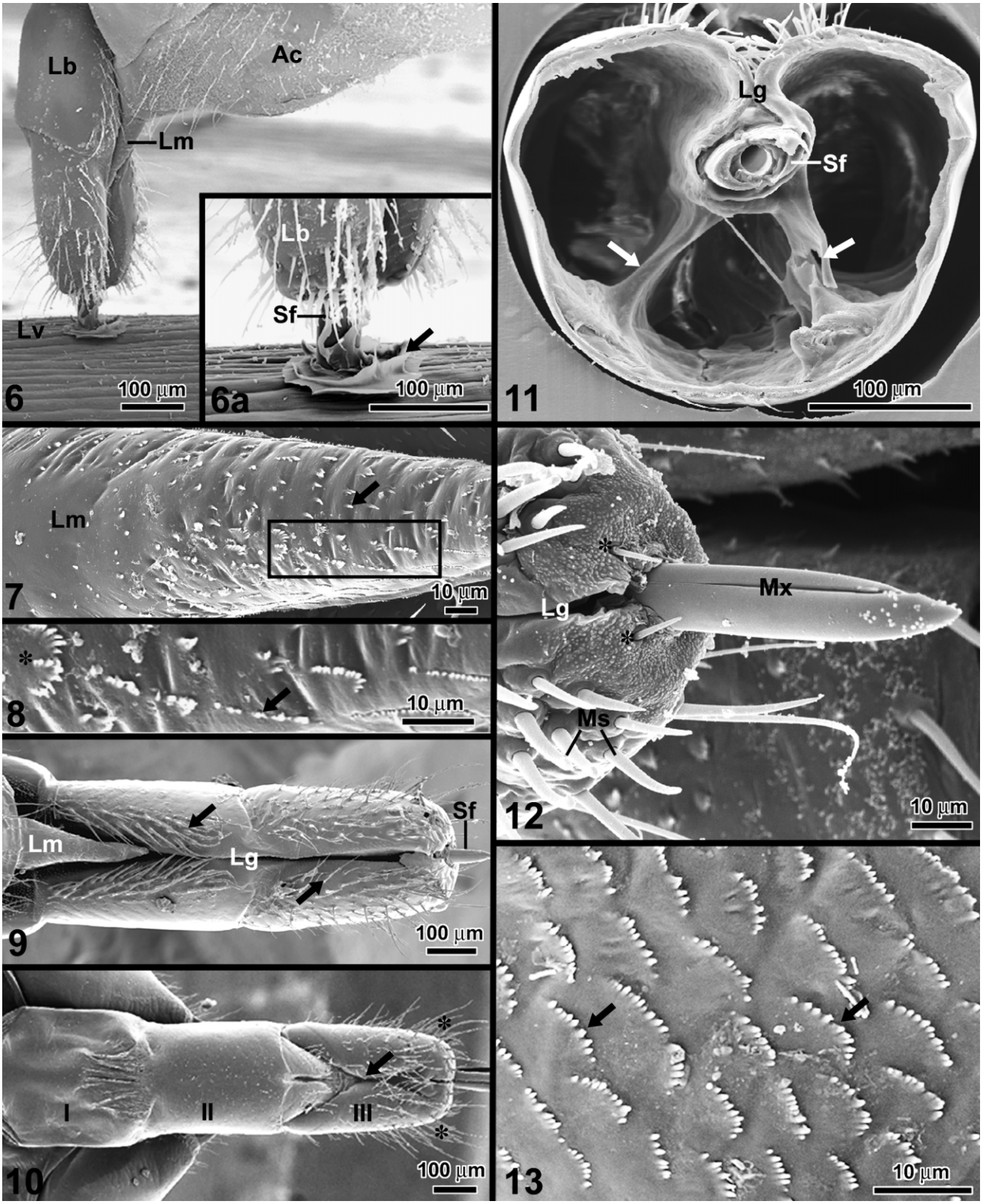
Figs. 1–5. Fig. 1: Light micrograph of glassy-winged sharpshooter adult feeding on the abaxial surface of a sunflower leaf vein. Fig. 2: Light micrograph of head portion of a cleared GWSS exuvium showing labium (Lb) encasing the mandibular (*) and maxillary (arrow) stylets. Fig. 3: Light micrograph of sunflower stem section showing two branched salivary sheaths (Ss) one of which terminates in xylem (Xy) vessels. Pp = pith parenchyma. Fig. 4: Light micrograph of sunflower stem section showing branching of salivary sheath (Ss) and encasement of the xylem (Xy) elements. Fig. 5: Light micrograph of sunflower stem section showing a branched salivary sheath (Ss) penetrating the pith parenchyma.

triangular spines of < 5 μm in length (Fig. 7) that occur singly or are arranged into clusters. Some of the clusters are in a straight line while others have a palmate shape (Fig. 8).

3.3. Labium

The labium (Lb) or rostrum is composed of three segments each having a ventral groove, the labial groove (Lg), that encases the stylet fascicle (Sf) (Figs. 2, 6, 9, and 10). The terminal segment has a fold on the dorsal surface giving this segment an overall appearance of an elongated fused bi-lobed structure (Fig. 10). The labial groove is essentially a fold in the exoskeleton of the labium which is strengthened along its length by two apodemes arising from the inner dorsal surface of the labial segments (Fig. 11). Most of our observations revealed that the stylet fascicle was positioned off center in the labial groove as shown in Fig. 11. Whether this is the case in the living insect is unknown.

Sensilla cover most of the surface of the terminal segment of the labium while the proximal and middle segments bear sensilla only along the labial groove (Figs. 6 and 9). All are hair-like, without pores, and are of the mechanosensory-type. No pit or dome-shaped sensilla were discerned on the outer surface of the terminal labial segment (Fig. 12). The sensilla along the labial groove of the middle and proximal segments of the labium and on the terminal segment are slender, slightly curved, directed towards the distal end of the labium, and range from 80 to150 μm in length. The longer sensilla are mainly located on the terminal labial segment. There are also shorter more robust sensilla that are located in and around the membranous end of the terminal segment close to where the stylet fascicle exits from the labium (Fig. 12). Depending upon the stage of the insect, these smaller sensilla range in length from 10 to 20 μm. The number of sensilla also varies with the age of the insect, with about 16 in the early instars and increase to an average of 30 on the adult labium. Two of the shortest sensilla are located on either side of the labial groove, immediately ventral to where stylet fascicle exits the labium and were often observed to be in contact with the stylets. The exposed surface of the labial groove ventral to where the stylet fascicle is held bears clusters of what may be peg sensilla that are arranged in scale-like rows (Fig. 13). Next to these clusters, lining the innermost surface of the labial groove, are numerous multi-lobed structures (Ml) having a



Figs. 6–13. Fig. 6: SEM of the portion of the anteclypeus (Ac), labium (Lb) and labrum (Lm) of a cast exuvium from a nymph that anchored the stylets into a leaf vein (Lv) before molting. (a) Enlargement of above showing the tip of the labium (Lb), the stylet fascicle (Sf), and the salivary sheath flange (arrow) around the fascicle and on the surface of the leaf. Fig. 7: SEM of the ventral outer view of triangular-shaped labrum (Lm) showing short surface spines (arrow). Fig. 8: SEM of the enlarged view of above outlined box showing clusters of pegs arranged in rows (arrow) and into palmate structures (∗). Fig. 9: SEM of the ventral view of labrum (Lm) and labium showing the surface arrangement of mechanosensory sensilla (arrows) along the labial groove (Lg) and a partial extension of

palmate appearance (Fig. 14). Somewhat similar, but larger and flatter structures are also present where the groove opens at the end of the terminal segment (Fig. 15). Both the pegs and the lobes of the palmate structures are directed toward the distal end of the terminal labial segment and are more numerous per unit of area in the adults than in the labia of the earlier instars.

3.4. Mandibular stylets

The mandibular (Md) stylets are located on each lateral side of the maxillary stylets. They are crescent-shaped in cross-section and thus form a groove for positioning of the maxillary stylets. The stylets taper to sharp points and are elaborately sculptured at the tips and along the borders (Figs. 16–19). Each stylet is manipulated by two sets of retractor and protractor muscles (not shown). When measured from the fascicle base to the anterior tip, the average length of a mandibular stylet of first instar nymphs is about 325 μm ($n = 12$, range = 259–366) as compared to about 810 μm in the adults ($n = 12$, range = 657–962).

The sculpturing is most noticeable at the extreme tip of each mandible. On the medial surface of each stylet tip is a series of cup-shaped flanges (Fl) (Figs. 17–20). The flanges are most prominent at the tip of the stylet and become reduced in height in a step-wise fashion as they extend cephalad to a point where they eventually disappear near the center of each stylet. The stylet surface between the flanges is covered with a field of small papillae < 0.2 μm in diameter (Figs. 18 and 20). To each lateral side of the flanges, ventral and dorsal to the maxillary stylets, there is a row of projections that may aid in the positioning of the maxillary stylets within the groove formed by the mandibular stylets (Figs. 17–20). On the ventral surface towards the tip of the adult stylet, the projections are slender, finger-like, and have pointed tips (Fig. 19). These finger-like structures are grooved where they are attached to the mandible, giving the impression that they may possess flexibility or articulation. Following these projections proximally, they gradually flatten and become more tab-like in structure (Fig. 16). On the whole, the lateral projections are much wider, flatter and less numerous on the nymphal stylets. However, as the insect proceeds through its nymphal instars, the projections become more slender and more numerous, apparently associated with the lengthening of the stylets.

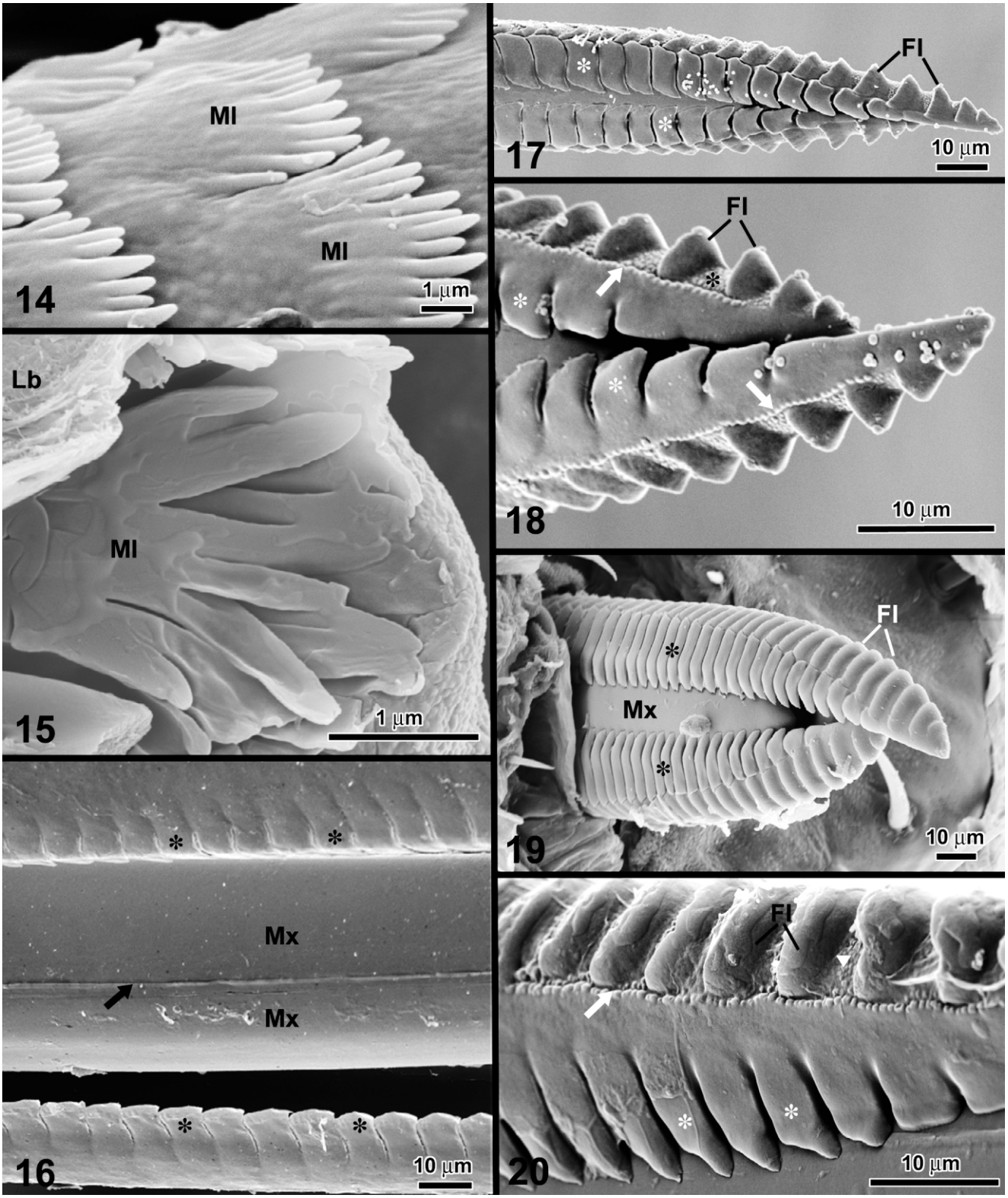
On the dorsal side of each mandibular stylet, the side closest to the head of the insect, the rows of projections have a different shape than those found on the ventral surface. These rows of lateral projections arise at the level of the third flange and begin as short, tab-like protuberances (Figs. 18 and 20). Along the edge near the stylet tip where these projections abut to the flanges, there is a row of papillae that are about twice the size of the papillae covering the surface between the flanges. As the row extends back toward the stylet base, the projections become longer and more pointed and then gradually flatten into rectangular, tab-like structures similar to the projections on the ventral surface of the stylet as shown on Fig. 16. Like the structures on the opposing side, the lateral projections are also less numerous, wider, and flatter in shape on the mandibles of the nymphs.

3.5. Maxillary stylets

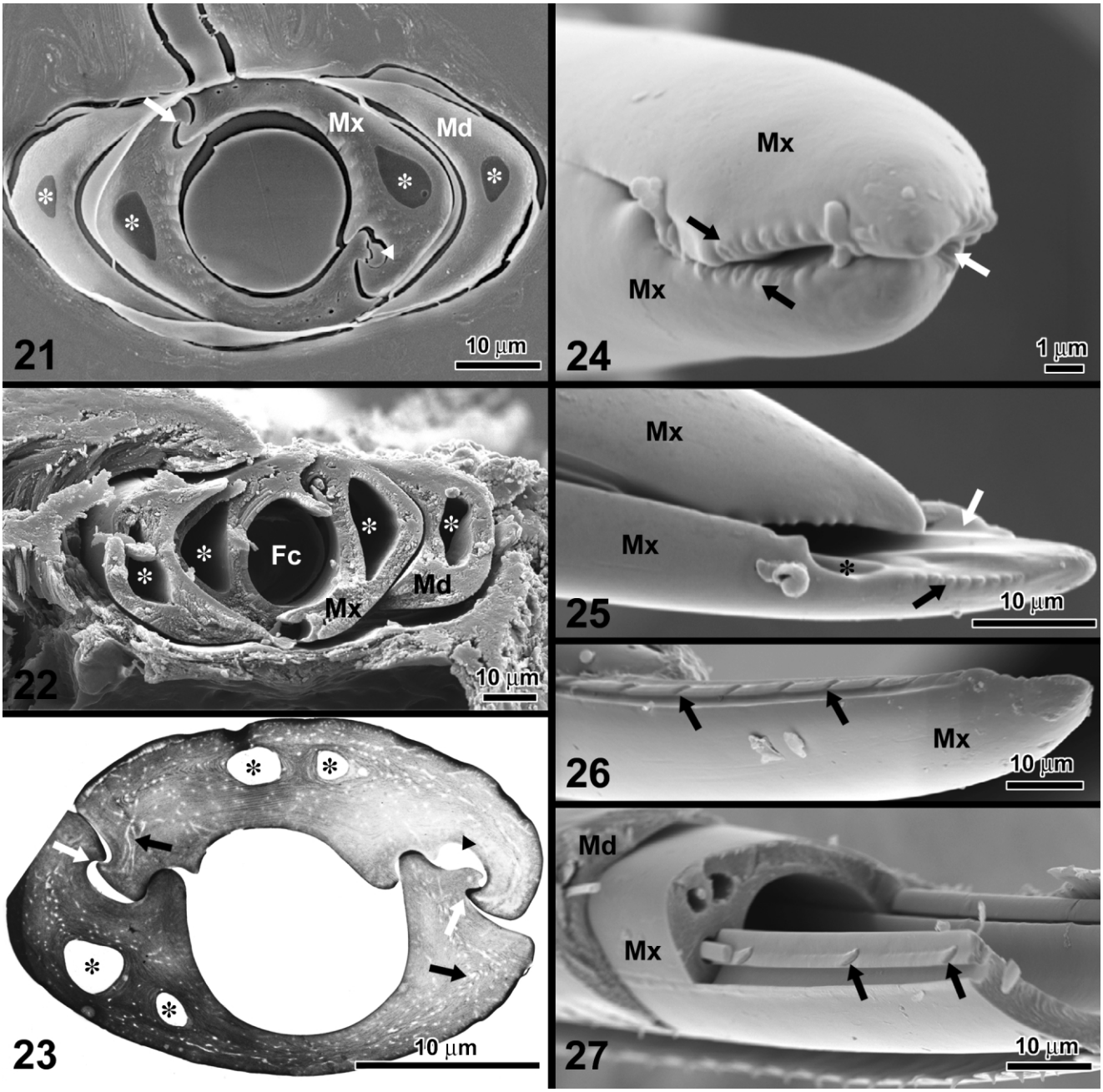
The two maxillary stylets (Mx) in cross-section are semi-circular along their length and interlock with each other (Figs. 21 and 23), thus forming a smooth hollow tubular structure that has a noticeable dentition near the tip of each stylet (Figs. 24 and 25). These denticles are slightly raised, round structures with depressed top surfaces and are arranged in a single rows of 7–9 on each side of the opposing stylet tips. Whether these structures were supplied with sensory neurons was not determined, but their position and shape are suggestive of pit receptors. The two joints, which attach each maxillary stylet to its mate are similar to that of a mortise and tenon type of joint (Figs. 21 and 23) and appear to run the length of the stylets except for a small area on the left side of the tip where the joint terminates before approaching the denticles (Fig. 25). The joint on the opposite side of the stylet bears the salivary canal (white arrow) which has a terminal opening between the denticles and the extreme end of the stylets (Figs. 24 and 25). Although each maxilla is similar in shape and dimension, the interlocking joints of the stylet are not located on a dorsal–ventral plane of the fascicle, but are canted off the midline from about 15° proximally to nearly 30° at the distal end. The length of stylets during the first instar average about 545 μm ($n = 6$, range = 483–620 μm) and reach 1235 μm ($n = 6$, range = 1141–1364 μm) by the adult stage.

On each side, along the interlocking surfaces of the maxillary stylets, there are series of oval-shaped nodes and corresponding indentations that suggests that these structures may function as a rachet device for positioning the stylets in apposition to each other (Figs. 26 and 27). We noticed that the two maxillary stylets were often off-set which formed an opening much larger than the small opening formed at the end the maxillae when they were perfectly matched in length. When the stylets were mismatched in length, the longer stylet tended to curve inward towards the midline (Fig. 26).

the sytlet fascicle (Sf). Fig. 10: SEM of the dorsal view shows three-segmented labium (I–III), mechanosensory sensilla (*), and the folded appearance of the terminal segment (arrow). Fig. 11: SEM of the cross-section through second labial segment showing the labial groove (Lg) containing the stylet fascicle (Sf) and supported by two apodemes (arrows). Fig. 12: SEM of the terminus of third labial segment showing clusters of mechanosensory sensilla (Ms) in the membranous portion of the labium. Asterisks denote the short sensilla on each side of the labial groove (Lg) next to maxillary stylets (Mx). Fig. 13: SEM of the surface of the labial groove above where stylet fascicle is retained. Arrows denote clusters of peg-shaped sensilla.



Figs. 14–20. Fig. 14: SEM of the inner surface of labial groove near the middle of the terminal labial segment showing multi-lobed structures (Ml) located below where stylet fascicle is retained. Fig. 15: SEM of the structures similar to those in Fig. 14 except they are flatter, have fewer lobes, and are located just inside the labial groove where it opens on the terminal labial segment (Lb). Fig. 16: SEM of the tab-like configuration of the lateral mandibular projections (*) that encase the interlocked maxillary stylets (Mx) proximal to the tip of the stylet fascicle. Arrow shows joint between the two stylets. Fig. 17: SEM of the lateral scale-like projections (*) and flanges (Fl) on the ventral side of the nymphal mandibular stylets. Fig. 18: SEM of the opposite side of the above showing the papillae (black *) between flanges (Fl) and also a row of papillae (arrows) between lateral projections (white *) and the flanges. Fig. 19: SEM of the ventral side of an adult mandibular stylet with the finger-like lateral projections (*) and flanges (Fl) encasing the maxillary stylets (Mx). Fig. 20: SEM of the dorsal view of the adult mandibular stylet showing the wider and straighter lateral projections (*), flanges (Fl) and a row of papillae (arrow) below the flanges (arrow head).



Figs. 21–27. Fig. 21: TEM cross-section through the stylet fascicle. The section shows mandibular (Md) and maxillary (Mx) stylets, the interlocking joints between the maxillary stylets (arrow), salivary canal (arrowhead), and a single dendritic canal (*) within each stylet. Fig. 22: Similar view to the above except that it is a SEM of a fractured preparation at a site proximal to Fig. 21. The dendritic canals (*) are larger in both mandibular (Md) and maxillary (Mx) stylets. Fc = food canal. Fig. 23: TEM section through an area near the distial tip of the maxillary stylets showing the interlocking joints (white arrows), salivary duct (arrowhead), branched dendritic canals (*), and the associated network of smaller canals (black arrows). Fig. 24: SEM view of the tips of the maxillary stylets (Mx) showing the row of denticles (black arrows) on each of stylets and the opening to the salivary canal (white arrow). Fig. 25: Similar to the above except that the stylets (Mx) are displaced giving a view of where the interlocking groove ends (*) as opposed to the far side where the groove continues on to allow discharge of the saliva (white arrow). Fig. 26: Further displacement of maxillary stylets (Mx) showing an inward curving of lower stylet and a series of indentations (arrows) on one face of the surface that forms the interlocking joint. Fig. 27: SEM of a fractured stylet fascicle showing the mandible (Md), the maxillae (Mx), and the series of nodes (arrows) within the joint surface that oppose the indentations displayed in Fig. 26.

Ducts and canals are evident when the stylet fascicle is viewed in cross-section. Each mandible bears a large unbranched dendrite canal that runs the length of the stylet and is located centrally in the thickest portion of each structure (Figs. 21 and 22). Within each maxillary stylet towards the proximal end there also is a single medial, triangular-shaped duct containing the dendritic trunks of nerves that innervate the sensory receptors of the maxillae. This duct becomes rounded and smaller as it traverses the length of the stylet and bifurcates approximately one-third of

the distance from the distal end of each stylet. Near the tip of the maxillae these branches again divide into numerous small ducts which contain neurons that presumably communicate with sensory organs on the end of the maxillary stylet (Fig. 23).

The salivary and the food canals are formed by the interlocking maxillary stylets. The food canal (Fc) is centrally located and has a diameter of about 6.2 μm in first instar nymph and averages 20.8 μm in the adult sharpshooters. The singular salivary canal (arrowhead) appears as an additional groove to the inner side of one of the joints where the stylets interlock (Figs. 21 and 23). When the maxillary stylets are extended equally, there is an opening formed for the salivary canal at the distal end of the juxtaposed maxillae (Fig. 24).

3.6. Stylet penetration

In our laboratory, feeding by the GWSS nymphs, older than second instar, was located on the larger leaf veins and on the stems or petioles of at least 14 different host plants. Feeding by first and second instar nymphs was observed to be almost exclusively on the leaf veins and leaf margins of plants such as chrysanthemum, sunflower and eggplant which suggests that the xylem elements on the stems and petioles of these plants were deeper than could be accessed by the stylets of the nymphs at this stage of development. Tracking of the penetration route of the GWSS stylet fascicle within the plant tissues of mostly sunflower plants was conducted by observing where feeding was occurring. That area was then excised for further examination of where the salivary-sheath material was deposited during the probing/feeding activity.

Penetration of plant tissue by the stylet fascicle was observed to be mostly by a perpendicular insertion directly through the epidermal cells. Penetration was not observed through stomata. Penetration sites on the host plant were evidenced by the presence of salivary flanges on the surface of the epidermis which were continuous with the internal portion of the sheath. Examination of 175 GWSS salivary sheaths located in sunflower stems revealed that about 65% terminated within the xylem tissue. Thus, most of the sheaths (Ss) traverse a straight line intracellularly through the epidermis, cortical parenchyma, sclerenchyma fibers of the bundle cap, phloem tissue and finally terminate in the xylem (Xy) of the vascular bundles (Fig. 3). Other sheaths were found to be unbranched to the inner cortex and then branched one or more times. Some of these sheaths terminated in xylem tissues while others did not. Approximately 36% of all branched sheaths terminated within the parenchymatous tissue of the cortex or in the medullary rays between the vascular bundles (Fig. 4). Only about 5% of the sheath branches terminated in pith parenchyma (Fig. 5). Rarely did we find branches terminating in either the phloem or in the sclerenchyma fibers of the bundle cap. Branching was observed to occur on several planes relative to the insertion point, indicating that the directional control of stylet probing is not just one dimensional (Figs. 28 and 29).

The sheaths were wider at the point of plant penetration, presumably to the depth of the mandibular stylet insertion, and became narrower at the point of branching. The point where the sheath width was narrowest correlates with maximum possible insertion length of the maxillary stylets. If a salivary sheath was observed to terminate in the xylem, it often spread out to surround one or more vessel elements (Fig. 4). Similar terminal sheath enlargements were not found to be present in parenchymatous tissues.

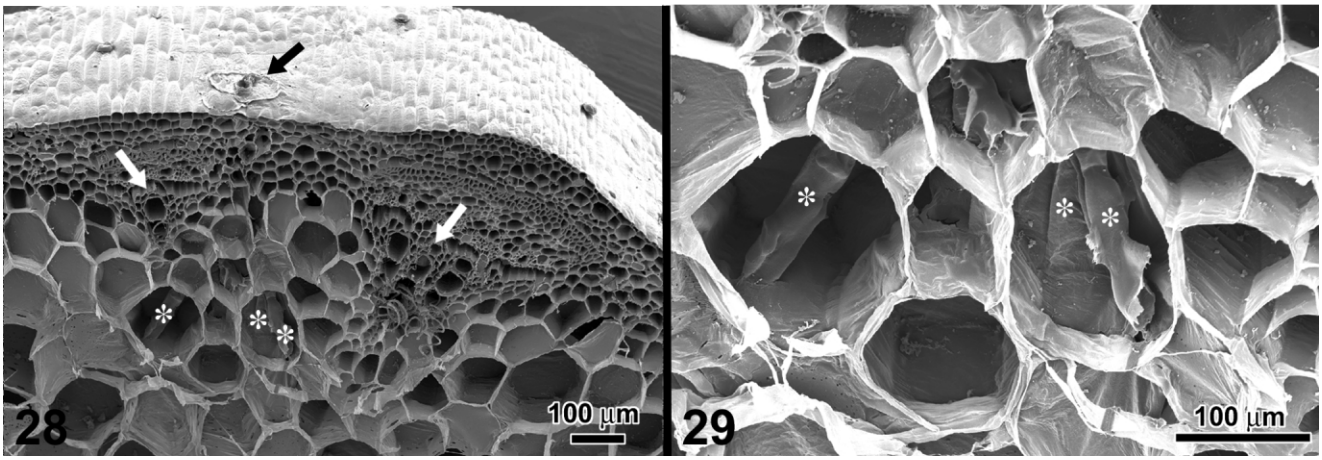
The length of the salivary sheaths varied with respect to depth of the xylem elements in a specific area of the plant (e.g. leaf vs. stem) and evidently with probing activity that terminated in non-xylem areas. The deepest penetration (ca. 1075 μm, $n = 3$) that was measured was that of adult feeding probes that ended outside of xylem tissues. Other measurements terminating in or near the xylem elements averaged ca. 930 μm ($n = 25$).

4. Discussion

The gross morphological features of the mouthparts of *H. coagulata* are similar to what have been reported for other homopterans (Cobben, 1978; Backus and McLean, 1982, 1985; Backus, 1985; Backus, 1988; Mora et al., 2001). The three-segmented labium, bearing a moderate number of mechanosensory hair-like sensilla and used in the perpendicular orientation of the stylet fascicle to the plant surface, is a typical pattern for many auchenorrhynchan species (Backus, 1988). While the hair-like sensilla most likely function in the detection of plant surface cues, it is venturesome to assign a sensory function to the clusters of peg- and multi-lobed structures located in the labial groove without TEM documentation. These latter structures bear an outward resemblance to the multi-lobed sensilla which have been detected on the outer labial surface of the leafhopper, *Peregrinus maidis* (Ashmead) (Backus, 1985). Lacking a sensory function, these structures could be used simply to rid the stylet fascicle of plant and salivary sheath debris during withdrawal following penetration.

The lack of chemosensory-type sensilla on the tip of the labium is apparently an unusual characteristic of this species since most auchenorrhychans studied thus far have some variation of porous sensory receptors (Foster et al., 1983; Backus, 1988). The GWSS has an exceedingly wide host range for feeding (Blua et al., 1999; Hoddle et al., 2003). It is tempting to speculate that an insect with the ability to feed on at least 100 documented hosts would not require extensive chemical cues emanating from the plant surface since such information would likely be extremely diverse. Thus, the need for chemosensory organs on the terminus of the labium of a generalist phytophagous insect such as the GWSS may be diminished.

The sculpturing on the apical surface of heteropteran mandibular stylets has been linked to the stabilization of the maxillary stylets and has been considered to be an



Figs. 28 and 29. Fig. 28: Sectional view through a cowpea leaf showing a salivary flange marking the site of penetration and salivary flange (black arrow) and three branches of the salivary sheath (*) that entered between the medullary rays (white arrows) and terminated in the parenchyma. Fig. 29: Enlarged view of the above micrograph showing the fine structure of the terminal branches (*) of the salivary sheath terminating in two parenchyma cells.

adaptation for holding on to prey or host tissues, thus aiding the mandibles to serve as a fulcrum for the movement of the maxillae (Cobben, 1978; Cohen, 2000; Wheeler, 2001). It is easy to visualize how the cup-shaped flanges on the tips of the mandibular stylets could provide stability for the maxillary stylets once they are embedded into the plant tissues. This stabilizing function of the mandibles may be enhanced by embedment in solidified sheath material since it appears that the salivary secretion extends from the site of insertion to the plant tissue where feeding occurs. Along with the secretion of a salivary flange around the exposed portion of the stylet fascicle at the site of plant penetration, these cup-like structures on the mandibles may also function in aiding the insects to exit from their exuviae during ecdysis. We have observed that all cast exuviae of at least the last three nymphal instars of the GWSS were also anchored to the host via insertion and spreading of the mandibular stylets into the plant tissues.

The structure of finger- and tab-like projections on the lateral fringes of the mandibular stylets yield the impression that they are flexible and may be involved in the directional movement of the maxillary stylets in the longitudinal plane of the insect. It would be informative to learn whether there is communication between dendrites located within the internal canals with these structures on the lateral fringes of the mandibles. Since we have observed that branching of the salivary sheaths can occur in different planes, movement of the maxillary stylets tangential to, or on the longitudinal axis of the insect could be aided directly by a physical manipulation of these projections or indirectly by yielding information of a proprioceptive nature upon deflection. Apparently, stylet movement in the transverse direction in related cicadellid species is accomplished by retraction of one of the mandibulary or maxillary stylets so that pressure on the leading end of the other stylet causes a deflection in the direction of the incurved tip (Pollard, 1969; Cobben, 1978).

Sogawa (1973) observed that the mandibles of leafhoppers were generally 74–79% as long as the maxillary stylets and that the mandibles of planthoppers were nearly as long as maxillae (93–99%). Our measurements show that the first instar GWSS nymphs have mandibles that are about 60% as long as the maxillae and increased to about 65% in the adult stage. The overall length of the maxillary stylets of first instar nymphs is probably the determining factor for feeding sites on plants such as the sunflower. The depth of the xylem elements within the sunflower stems of plants 30–45 cm high is more than twice the total length of the stylet fascicle, whereas in leaf veins and vein tracheids on the periphery of the leaves these vessels are located much closer to the surface. Further, since our measurements were made from the base of the fascicle attachment and not from the tip of the labium, the effective extrusion length of the fascicle would logically be limited by how much the labium could be compressed. Interestingly, Pollard (1968) noted that stylet penetration of fifth instar nymphs of *Eupteryx mellissae* Curtis into host plants was deeper than the adult stage even though the adults had the longer maxillary stylets. He suggested that this may be an adaptation for a secure anchoring of the exuvia during molting of that particular nymphal stage.

The strictly smooth outer and inner surfaces of the maxillary stylets of *H. coagulata* is apparently typical of a salivary sheath-producing phytophagous homopteran insect that feeds on the plant fluids contained in the vascular tissues (Cobben, 1978). In contrast, insects employing a 'lacerate and flush' feeding strategy (Miles, 1958; Smith, 1985) often possess maxillary stylets with some type of modification which effects the disruption of the host tissues.

The salivary sheath of vascular feeding homopterans has been identified as a lipoproteinacious material that is secreted and solidifies around the stylets during penetration of the host plant (Miles, 1968, 1972). Various suggestions have been made as to the function of the salivary sheath

during feeding that include support for the stylets, lubrication, sealing vascular tissues as a protection against leakage, and as an aid to directional control (Miles, 1964, 1972; Pollard, 1973). Because the flow of fluid in the xylem vessels is under a negative pressure (Baker, 1984), it would seem likely that the sheath would be important for insects tapping into these areas to prevent cavitation in the vessels and leakage from the con-joined maxillary stylets. There are two observations in this study that tend to support the protection against leakage hypothesis. The GWSS produces a salivary flange, that is apparently tightly sealed against the labium during feeding and is continuous with the internal portion of the sheath. Plus, there is additional salivary sheath material secreted in and around the xylem elements not found in other areas where probing terminated, indicating that initial the penetration or the repeated penetration of adjacent vessels probably requires some amount of patching via the salivary secretion.

The presence of prominent dendritic canals within the mandibulary and maxillary stylets indicate that the dual innervation of the fascicle is extensive and probably involves a proprioceptive function. The studies of Forbes and Raine (1973), Wensler (1974), Backus and McLean (1982), and Foster et al. (1983) clearly show the presence of sensory receptors within the main stylet canals of several related homopteran species. Our observations on sections through the tips of the maxillary stylets show that there is a complex network of small canals containing neurons, indicating that this area of the stylet has an important sensory function. Backus and McLean (1984) report that the precibarial sensilla of the cicadellid, *Graphocephala atropunctata* (Signoret), are gustatory chemosensilla and are involved in determination of the quality of internal plant fluids. To date, evidence for the presence of chemoreceptors associated with either the mandibulary or maxillary stylets for plant-sucking insects is lacking, but the internal and external features on the distal end of the GWSS maxillary stylets are suggestive of other sensory functions besides proprioception.

In summary, this study has revealed a number of intriguing aspects related to structure and how the mouthparts of the GWSS function. While the gross configuration of the mouthpart complex resembles what has been described for other salivary sheath-producing homopterans, the previously undescribed accessory structures associated with labrum, labium and stylet fascicle provide compelling impetus for further studies. For example, electrophysiological and additional in-depth TEM studies will be required to determine a function for the curious hand-shaped structures located in the labial groove. Further, while we were unable to obtain samples of the stylets inserted within host tissues by an actively feeding insect, we expect that such information will be forthcoming and with the data gained in this study, a clear picture of GWSS feeding process can be assembled.

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